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FINAL REPORT

THE BIOSPHERE AS A POSSIBLE SINK FOR CARBON MONOXIDE EMITTED TO THE ATMOSPHERE

Prepared for:

COORDINATING RESEARCH COUNCIL
NEW YORK, NEW YORK

Contract CAPA-4-68(2-68)

and

NATIONAL AIR POLLUTION CONTROL ADMINISTRATION
ENVIRONMENTAL HEALTH SERVICE
PUBLIC HEALTH SERVICE
DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
DURHAM, NORTH CAROLINA

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This report describes studies conducted to determine the possibility of certain elements in the biosphere serving as sinks for carbon monoxide emitted to the atmosphere by various human activities. This was accomplished by exposing test samples to 100 ppm carbon monoxide (static experiments).

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I INTRODUCTION AND BACKGROUND

The current annual rate of carbon monoxide emission into the earth's atmosphere due to urban activities has been estimated to be 2.1×10^{14} grams. On the basis of this emission rate, the current average atmospheric concentration of carbon monoxide, 0.2 ppm, should be increased each year by 0.043 ppm, and a doubling of the present concentration could be expected within four to five years. However, carbon monoxide atmospheric concentrations have remained essentially constant over the past ten to twenty years, which suggests that some form of carbon monoxide sink or pool must be operating. The existence of such a sink, however, has not been clearly demonstrated.

The mechanism of disappearance of large quantities of carbon monoxide from the atmosphere is largely a matter for conjecture. Certain elements of the biosphere seemingly have the potential to act as a carbon monoxide sink, but none has been demonstrated to do so. Hemoglobin, myoglobin, and certain of the cytochromes are known to bind carbon monoxide at iron binding sites, but this bound carbon monoxide is released to the atmosphere once again when the molecules are degraded. Besides releasing bound carbon monoxide, conversion of the porphyrin ring of heme to bile pigment during hemoglobin degradation is accompanied by cleavage and oxidation of the α-methyne bridge carbon to carbon monoxide.

Metabolism, rather than binding, might provide a partial answer to carbon monoxide disappearance. Dr. Wallace Fenn, University of Rochester, compiled a table of known rates of carbon monoxide utilization from various sources in the literature. This table was presented at the New York Academy of Sciences in January 1970.

	Rate*
Sea urchin egg	6.1
Frog heart	3.4
Frog muscle	1.2
Rat heart	2.9
Mice	6.8
Dog	6.3
Man	1.0
Algae	3.9

If one assumes an average weight per man of 150 pounds and the world population at 3.4×10^9 persons, this utilization could account for 1.3×10^{14} grams of carbon monoxide per year. However, Fenn noted that production of carbon monoxide by hemoglobin degradation is reported to be 30 times as great as the carbon monoxide burned. Thus, the likelihood of tissue metabolism being a large sink for carbon monoxide seems small.

Another major sector of the biosphere that may act as a carbon monoxide sink is the plant kingdom, including the seed plants, ferns, mosses, algae, and microorganisms. Higher plants would seem likely as prospective removers of carbon monoxide, for they are ideally structured for removing low concentrations of gases from the atmosphere. According to Rabinowitch,[8] land plants use about 7×10^{10} tons of carbon dioxide every year in photosynthesis. If land plants removed 1 gram of carbon monoxide for every 350 grams of carbon dioxide used, land plants could be a sink for which we are searching. Since structural analogs often can fit the same receptor and be slowly utilized as substrates, this is certainly feasible. There is speculation that cytochrome oxidase, a common

---

* Rate in cu mm/gram/min at 37° and 1 atm assuming:

$Q_{10} = 2.0$; COHb = 10%: rate proportional to P_{CO} (atm) and to body weight.

component of the respiratory chain of plants and animals, can both use and be inhibited by carbon monoxide simultaneously.[1] The literature on the effects of carbon monoxide in low concentrations on plants, however, is too sparse to suggest many answers.

Microorganisms as well as higher plants possess the potential for removing carbon monoxide. Jones[2] noted that carbon monoxide disappeared while passing through certain soils, probably due to the presence of microorganisms. Kluyver,[4] Yagi,[12] and Jones[2] found that carbon monoxide could be utilized by certain types of bacteria. Kistner[3] reported that Hydrogenomonas carboxydovorans could oxidize carbon monoxide to carbon dioxide:

$$2CO + O_2 \rightarrow 2CO_2 + 123.5 \text{ kcal}$$

Marine and fresh water algae also are possible users of carbon monoxide. Rabinowitch postulated that sea plants use approximately 5.7×10^{11} metric tons of carbon dioxide in photosynthesis each year.[8] Thus, if sea plants absorbed 1 gram of carbon monoxide for every 2,700 grams of carbon dioxide absorbed, this would account for all of the carbon monoxide missing annually from the atmosphere. The metabolic processes of these plants are essentially the same or similar to those of the higher plants, and therefore could also be expected to remove carbon monoxide from their water environments if land plants are capable of removing significant amounts of carbon monoxide from the atmosphere.

Carbon monoxide has been found in algae, but evidence indicates that it is a metabolic product rather than an accumulation from the surrounding environment. The unicellular algae, Cyanidium evolved carbon monoxide during the synthesis of the bile pigment phycocyanobilin.[10] Nereoceptis, a Pacific Coast kelp, contained up to 12% carbon monoxide in its bladders.[6] Egregia menzies, a brown algae, contained carbon monoxide in its pneumatocysts. Homogenates of fresh Egregia tissue

incubated in potassium phosphate buffer evolved carbon monoxide in a heat stable reaction.[2]

Aside from the presence of carbon monoxide in pneumatocysts of certain algae, carbon monoxide has been detected in the pneumatophores of siphonophores and in the float of the Portuguese Man-of-War. Nanomia propel themselves through the deep scattering layer of the ocean by the expulsion of carbon monoxide. Carbon monoxide is maintained in their pneumatophores at concentrations exceeding 90%.[7] Physalia contain up to 13% carbon monoxide in their floats. In the case of Physalia, it has been postulated that this initial concentration of carbon monoxide serves to inflate the float and is later replaced by air through diffusion and exchange. L-Serine has been shown to be the source of the carbon monoxide.[11] (It is interesting to note that barley leaves incorporate carbon monoxide mainly in the serine fraction.[5])

Thus, several species in the ocean are metabolically active in relation to carbon monoxide. Depending on whether metabolic balance is toward production or utilization of carbon monoxide, the ocean could serve as a sink or a source of carbon monoxide. From measurements of carbon monoxide concentrations in the atmosphere and surface waters of the North Atlantic Ocean, Swinnerton[9] concluded that the ocean is a source rather than a sink for carbon monoxide, because the surface waters appeared supersaturated with respect to the partial pressure of carbon monoxide in the atmosphere.

The purpose of this research project was to survey various elements of the biosphere for their capabilities to remove carbon monoxide from the atmosphere to determine if a sink does indeed exist in the biosphere, the elements of the biosphere that make up this sink, and their quantitative capacities to remove carbon monoxide.

II SUMMARY AND CONCLUSIONS

This report describes studies conducted to determine the possibility of certain elements in the biosphere serving as sinks for carbon monoxide emitted to the atmosphere by various human activities. This was accomplished by exposing test samples to 100 ppm carbon monoxide (static experiments). The results show:

1. Nonsterile soil depleted carbon monoxide rapidly from test atmospheres containing initial concentrations of 100 ppm carbon monoxide. This effect was enhanced by increasing temperatures and eliminated by steam sterilization, indicating that heat-labile biological mechanisms were involved. The minimal experimental depletion rates demonstrated theoretically could account for 2.06×10^{15} grams carbon monoxide per year on a worldwide basis.

2. Moistened vermiculite exposed to ambient air for several weeks depleted carbon monoxide from test atmospheres containing 100 ppm carbon monoxide. Sterilization eliminated this effect.

3. Carbon monoxide decreased in the atmosphere above plants of pepper, geranium, and barley growing in nonsterile support medium (soil, vermiculite). However, the role of higher plants as a possible carbon monoxide sink could not be adequately assessed because the plant effects, if any, were masked by those of the support media.

4. The effect of marine plants on carbon moroxide disappearance indicated a trend toward marine plant utilization of carbon monoxide at temperatures of 19.5°C but not at 10°C.

Thus, the results suggest that the microorganisms in the biosphere can serve as a carbon monoxide sink. Future work proposed includes the evaluation of soils from different locations as carbon monoxide sinks and the isolation of the organisms responsible.

III METHODS AND MATERIALS

Test Specimens

Support Medium

Plant support medium used in these studies was either a prepared soil mixture or vermiculite. The soil mixture consisted of commercially supplied sandy loam (55%) and Canadian Brand spaghum peat moss (45%) and 16-20-0 fertilizer (100 grams/cu yd). This mixture was moistened and stored in a sheltered place at ambient environmental temperature for the duration of testing. The same mixture was used for the entire series of soil experiments. Vermiculite was commercially supplied by Terralite and not moistened until placed in a greenhouse.

Plant Specimens

Hordeum vulgarum and Capsicum annuum were grown in a greenhouse for 6 to 7 weeks in soil or vermiculite in 16-inch-square fiber glass pans or 2.5-inch plastic pots. Plants were transferred to experimental environators at time of testing.

Pelargonium sections 3 to 6 inches in height were gathered from indigenous mature plants at a site approximately 0.25 mile from a freeway. These were placed in 2.5-inch pots with moistened vermiculite and allowed to remain in the greenhouse for approximately two weeks before testing.

Marine Specimens

Brown algae specimens (Cystoseira, Egregia, Macrocystis) were obtained from the Pacific Ocean off the southern California coast with seawater samples during March and tested within 24 hours of gathering. They were kept in cool,

filtered, recirculated seawater until transported for testing. The water temperature was 55°F or lower, to suppress the growth of bacteria in the seawater.

Apparatus

Biological Testing Apparatus

Four Germfree fiber glass environmental subunits (17½ by 17½ by 24 inches) with Plexiglas tops were housed in a Sherer walk-in growth chamber under 2500-2700 footcandles of light. Temperatures within the growth chamber were adjusted to provide the desired test temperatures inside the environmental subunits. Temperatures inside the subunits were monitored by means of cable thermometers fitted with air inlets and outlets (¼-inch tubing), sampling septums, and squirrel cage circulating fans. The air inlets were connected to a panel board of metering valves and Brooks rotometers. Nonreactive Teflon tubing and stainless steel fittings were used throughout.

To accommodate marine apparatus for testing of saltwater algae, two feed-through connectors were sealed in the Plexiglas lid of each environator. The test atmosphere was recirculated through the seawater containing the test algae at the rate of 0.1 cubic feet per minute by means of a diaphragm pump. Test specimens were contained in 11.5 liters of seawater held in 25-liter glass battery jars.

Gas Analysis Apparatus

A Loenco gas chromatograph equipped with a flame ionization detector was used for methane analysis. A 12-foot by 3/16-inch OD aluminum column containing a 60/70 mesh molecular sieve, Type 5A, preceded a short Ascarite column, which was in turn connected to a 12-inch by ¼-inch OD nickel catalyst bed. This was connected to the Loenco apparatus, which in turn was connected to a Hewlett Packard recorder. Stainless steel fittings were used throughout.

A Hamilton (1000-microliter) gas-tight syringe was used for all gas samples.

Experimental Procedures

Preparation of Small Environators

Environmental subunits were cleansed with a trisodium phosphate solution and thoroughly rinsed and dried before each experiment. During the preparation of the small environators, the walk-in environator air recirculation system was turned off to keep contamination from air to a minimum. Initially, a small ultraviolet light source was placed in each small environator for 15 minutes before flushing with the experimental gas mixture began. This was discontinued in later experiments.

Two liters of water, sterile or nonsterile, were placed in the bottom of each environator prior to insertion of test samples. After placement of specimens, the environators were sealed and flushed with 10 to 15 cubic feet of the test atmosphere (usually air containing 100 ppm carbon monoxide) while the squirrel cage fans were in operation. Environators not containing carbon monoxide were exposed to the normal atmosphere during the equilibration period. Preparation of the environators was the same for all experiments.

Support Medium Testing Procedure

Soil or vermiculite was placed in 8-inch by 8-inch by 2-inch Pyrex pans lined with 20-inch lengths of cheesecloth for experimental testing of the sterilized (versus nonsterilized) support medium. The pieces of cheesecloth acted as wicks and kept the support medium moist by drawing water as needed from the floors of the small environators. Containers to be sterilized were sealed in paper bags, autoclaved for at least 30 minutes (250^{o}F at 15 psi), and allowed to cool to room temperature. Support medium containers were then placed on the floor of the clean environmental subunits into which two liters of water had been poured. In the

case of sterilized support medium, sterilized water was used; with nonsterilized support medium, nonsterile water was used. The lids were then sealed and the small environators flushed with 100 ppm carbon monoxide.

Soil samples were tested at 22.5°C light (10 hours) and 17.8°C dark (14 hours) or 29.5°C light (10 hours) and 25°C dark (14 hours). Vermiculite was tested at 29.5°C light (10 hours) and 25°C dark (14 hours). Duration of experiments was three days or until carbon monoxide concentration reached a low level (usually less than 10 ppm). Small environators contained (1) unsterilized support medium plus 100 ppm carbon monoxide in balance air,* (2) sterilized support medium plus 100 ppm carbon monoxide in balance air, (3) unsterilized support medium plus ambient air, and (4) sterilized support medium plus ambient air. In the case of soil, these variables were tested simultaneously; in the case of vermiculite, some were tested sequentially.

Land Plant Testing Procedure

Hordeum, Capsicum, and Pelargonium were tested at 30°C light (10 hours) and 25.5°C dark (14 hours). Procedure and duration of experiments were the same as those described for support medium experiments. Environators contained (1) plants in support medium plus 100 ppm carbon monoxide in balance air, (2) support medium plus 100 ppm carbon monoxide in balance air, and (3) plants in support medium in ambient air. Other conditions were those described above.

Ocean Plant Testing Procedure

Three brown saltwater algae possessing floats, Cystoseira, Macrocystis, and Egregia, were supplied in fresh condition by Pacific Bio-Marine. These were tested in the presence of carbon monoxide (100 ppm) at two temperatures, 10°C and 19.5°C, both at cycles of 10 hours light and 14 hours dark. Because of the

* Balance air means that after the carbon monoxide was measured into the gas cylinder, the balance of the volume of the cylinder was filled with air.

limited cooling capacity of the walk-in chamber, lighting consisted of a row of incandescent bulbs above the environators. Algae were placed in 11.5 liters of aerated seawater (aerated by recirculation of the small environator contents) and exposed to 100 ppm carbon monoxide in balance air for two days. Algae were blotted and weighed immediately upon termination of the experiment. Environators contained 11.5 liters seawater plus 100 ppm carbon monoxide in balance air and (1) Macrocystis, (2) Egregia, (3) Cystoseira, or (4) no added specimen.

Gas Sampling Procedure

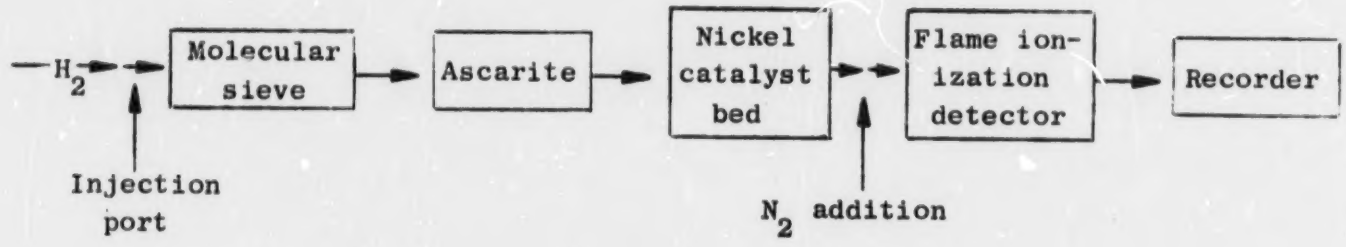
Aliquots (1 ml) of the test atmospheres were analyzed for carbon monoxide content by means of gas chromatography. The aliquots were withdrawn from the environmental subunits with a calibrated glass syringe by puncturing septums located on the sides of the small environators. The syringe was flushed twice with the sample gas before the sample was withdrawn.

Analytical Method

The analytical technique was based on the catalytic reduction of carbon monoxide to methane followed by flame ionization detection of methane. The method used has been described by Porter and Volman (Anal. Chem., 34, 748, 1962). Prior to reduction of carbon monoxide to methane, carbon monoxide was separated from other components of the sample gas by using a 12-foot by 3/16-inch OD aluminum column containing 60/70 mesh molecular sieve, Type 5A. Column temperature was 150°C, with hydrogen as the carrier gas (35 ml/min).

A short Ascarite column was inserted between the chromatographic column and the reduction reactor to remove carbon dioxide, which would otherwise disturb subsequent analyses. Reduction of carbon monoxide took place at 350°-500°C in a catalyst bed (12 inches by ¼ inch OD, filled with 60/80 mesh Chromasorb "W", and impregnated with nickel) via equilibration with a saturated solution of nickel

nitrate filtered and heated in oxygen for 20 hours at 400°-450°C. (It was found in later preparations of the catalyst that this temperature could be reduced.) The granules were then reduced in situ at about 350°C in a hydrogen stream to form the final catalyst. A diagram of the analytical system is shown below:



In addition, a purified nitrogen stream was added to the reactor outlet stream prior to the flame inlet. Dilution of the hydrogen stream in this manner increased the detection sensitivity several times. Using this procedure, the repeatability of the method over a two-day period is 110±0.9 ppm carbon monoxide SE (nine samples).

Performance Checks

To ensure that changing conditions within the system would not affect the accuracy of the method, standard gas samples were analyzed between each experimental sample in most cases. Interpolation of the two calibration gas readings then became the standard basis for comparison. Changes in reading values were correlated with slight temperature changes of the reactor column, and this was duly noted.

Empty sealed environators were filled and tested with 100 ppm carbon monoxide and balance air periodically between experiments to ensure that no leaks had developed in the small environators.

IV RESULTS

Support Medium Studies

Soil Studies

At 22.5°C in the presence of nonsterile soil, carbon monoxide was depleted from initial levels of about 100 ppm within 48 to 64 hours. See Figure 1. Rates of carbon monoxide depletion were essentially constant until concentrations reached 10-20 ppm. The average linear depletion rate was 2.2 ppm per hour in two experiments, and 1.7 ppm per hour in a third experiment, under a temperature regime of 22.5°C light and 17.8°C dark. In a separate experiment, rates did not decrease through three consecutive exposures of the same soil sample to approximately 100 ppm carbon monoxide (Exposure 1 = 3.6 ppm/hr, Exposure 3 = 4.1 ppm/hr). Rates of carbon monoxide depletion increased markedly at higher temperatures. Under a regime of 29.5°C light and 25°C dark, carbon monoxide depletion averaged 41 ppm per hour in three experiments, and 3.6 ppm per hour in a fourth experiment. See Figure 2. The cause of failure of depletion mechanisms in the latter experiment is not understood.

The capability of aliquots of the same soil sample to remove carbon monoxide under either temperature regime was destroyed by sterilization, as shown in Figure 3. Sterilized soil exposed to ambient air rather than air containing 100 ppm carbon monoxide caused a slight increase in carbon monoxide concentration. See Table 1.

Table 1

CONCENTRATION OF CARBON MONOXIDE DURING EXPOSURE OF SOIL TO AMBIENT AIR AT 22.5°C

	Carbon Monoxide Concentration (ppm)			
	Sterilized Soil		Unsterilized Soil	
Experiment	0 Hr	48 Hr	0 Hr	48 Hr
1	1.4	10.1	1.4	2.9
2	0	11.1	1.4	1.4
3	1.4	10.0	1.4	0

Figure 1

EFFECT OF EXPOSURE OF UNSTERILIZED SOIL TO 100 PPM CARBON MONOXIDE
AT 22.5°C LIGHT AND 17.8°C DARK ON CONCENTRATION OF CARBON MONOXIDE

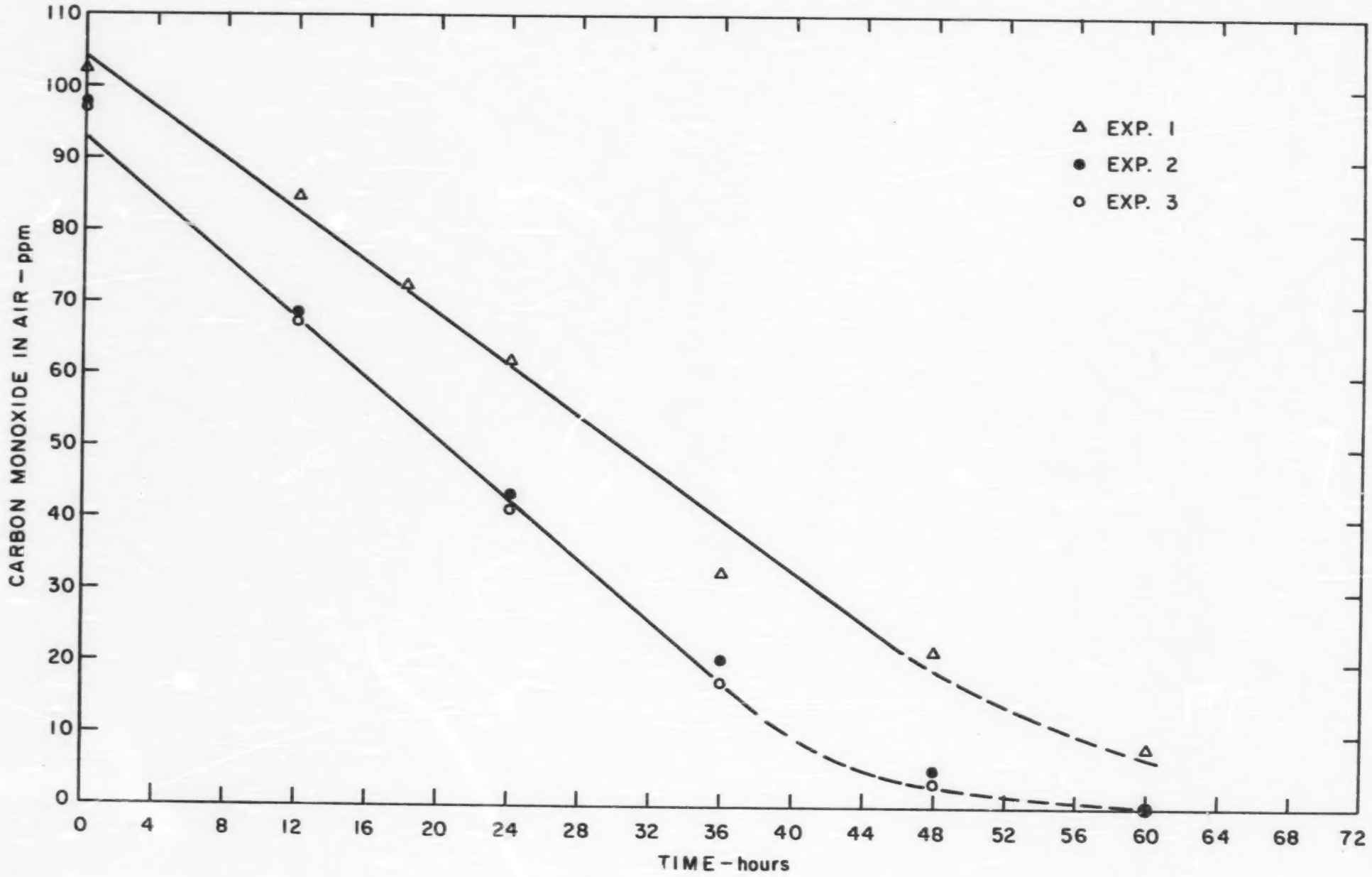


Figure 2

EFFECT OF EXPOSURE OF UNSTERILIZED SOIL TO 100 PPM CARBON MONOXIDE IN AIR AT 29.5°C LIGHT AND 25°C DARK ON CONCENTRATION OF CARBON MONOXIDE

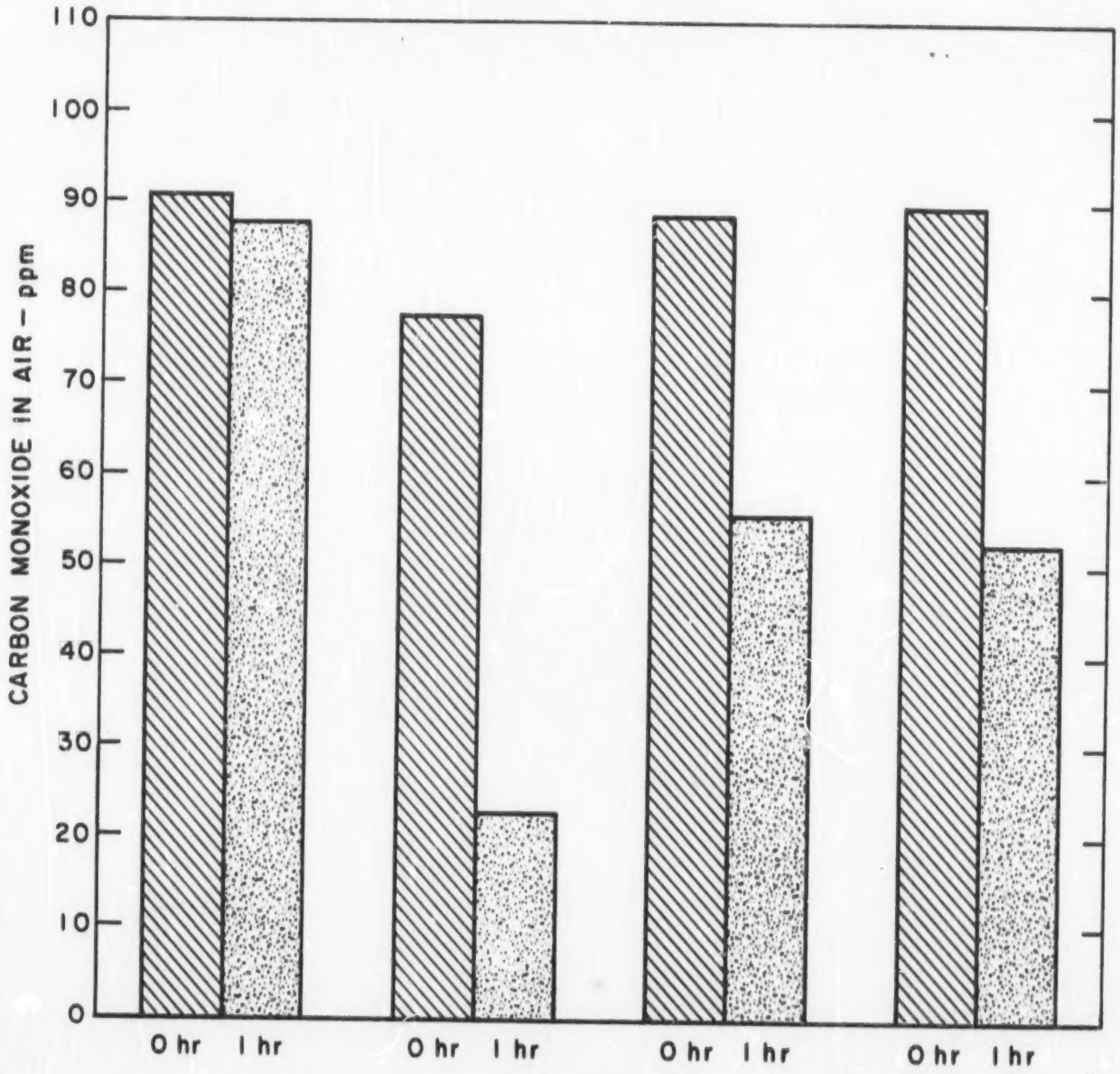
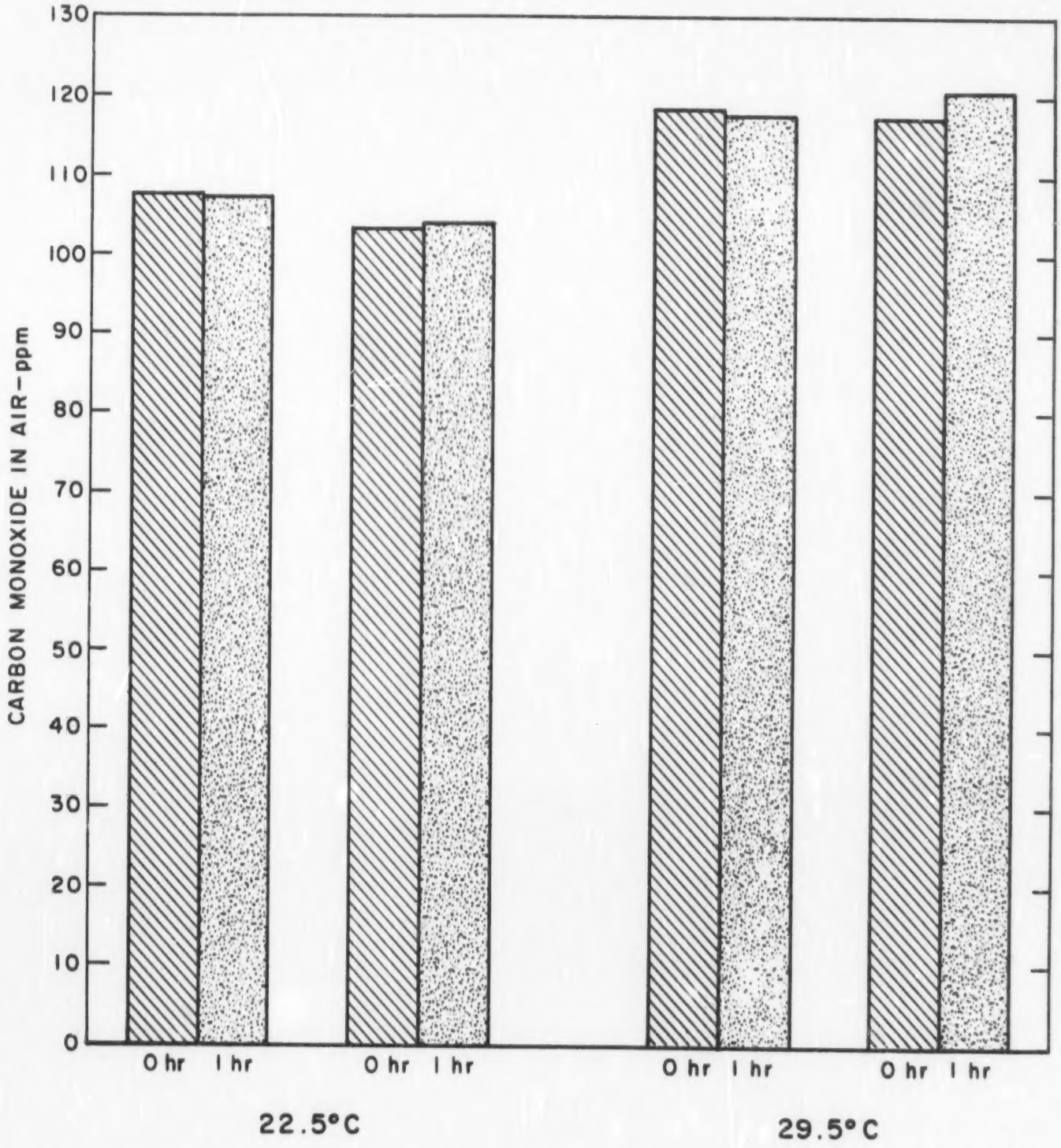


Figure 3

EFFECT OF EXPOSURE OF STERILIZED SOIL TO 100 PPM CARBON MONOXIDE AT 29.5°C LIGHT AND 22.5°C LIGHT ON CONCENTRATION OF CARBON MONOXIDE



Vermiculite Studies

Vermiculite, an inert support medium containing no organic constituents that might bind carbon monoxide, was moistened and left exposed in the greenhouse for several weeks. Periodically, the vermiculite was tested in the presence of 100 ppm carbon monoxide in balance air. Successive exposures resulted in increasing rates of disappearance. See Figure 4. Well-established colonies of algae and fungi were visible during this time on the vermiculite. Sterilization of the vermiculite followed by an exposure to 100 ppm carbon monoxide eliminated the disappearance of carbon monoxide in duplicate experiments. In the case of vermiculite, however, there was no apparent increase in carbon monoxide levels after sterilization, as with sterilized soil. Thus, carbon monoxide disappeared in the presence of both nonsterile soil and incubated nonsterile vermiculite. Sterilization eliminated this disappearance. Therefore, it is concluded that the mechanism of carbon monoxide disappearance is heat labile, and presumably biological.

Land Plant Studies

Carbon monoxide concentrations decreased from initial levels of 100 ppm in the presence of plants in support medium under both temperature regimes. See Table 2. Significant decreases, however, also occurred in the presence of soil support medium alone. Vermiculite support medium alone caused a decrease in one experiment and an increase in another.

At 22.5^{o}C, the rate of carbon monoxide uptake in the presence of pepper plants in soil was less than in the presence of soil alone. At 30^{o}C, however, the results were reversed--the rate of carbon monoxide uptake was greater in the presence of pepper plants in soil than in soil alone. In the case of both barley and geraniums in vermiculite at 30^{o}C, carbon monoxide removal was also greater in the presence of the plants than with the support medium alone, but this may have

Figure 4

EFFECT OF REPEATED EXPOSURES OF VERMICULITE TO CARBON MONOXIDE
IN A SEALED ENVIRONMENTAL CHAMBER
ON CONCENTRATION OF CARBON MONOXIDE

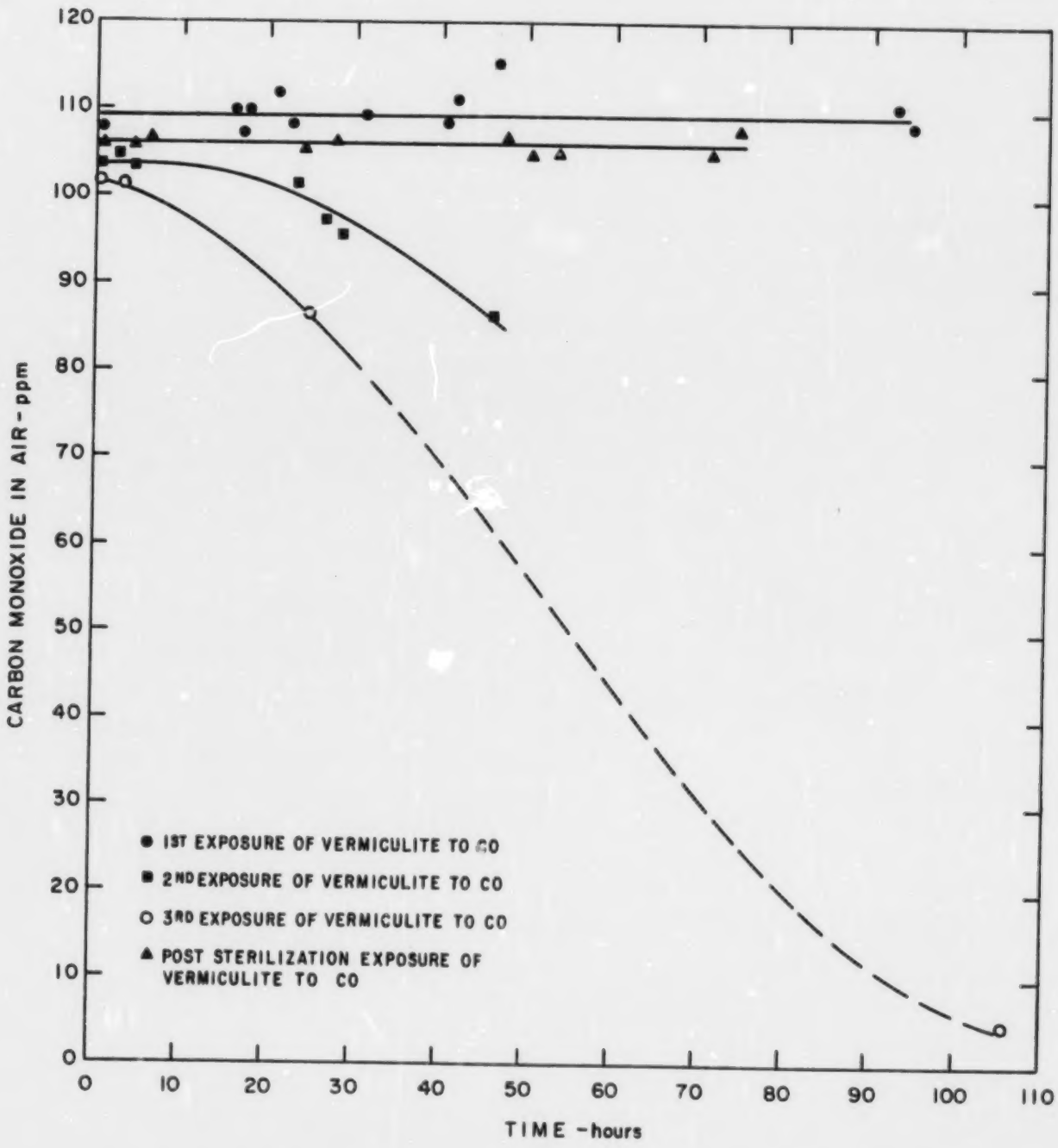


Table 2

EFFECT OF PLANTS ON CARBON MONOXIDE DISAPPEARANCE
AT 22.5°C AND 30°C

Specimen	Original Carbon Monoxide Concentration (ppm)	Light Temperature	Carbon Monoxide Concentration with Time (ppm) 0 Hr	24 Hr	36 Hr	48 Hr
Peppers in soil	100	22.5°C	112.2	92.2	--	74.0
Peppers in soil	0		1.5	2.4	--	4.9
Soil	100		111.5	80.8	--	53.5
Peppers in soil	100	30°C	110.6	12.4	7.1	--
Peppers in soil	0		2.3	5.7	5.0	--
Soil	100		108.3	40.3	14.1	--
Barley in soil	100	30°C	112.8	96.1	--	44.2
Roots of barley in soil	100		104.5	91.7	--	74.2
Barley in vermiculite	100		111.4	87.0	--	63.1
Vermiculite	100		108.0	106.6	--	115.7[a]
Geraniums in vermiculite	100	30°C	107.7	46.9	--	27.0
Geraniums in vermiculite	0		3.5	8.4	--	9.1
Vermiculite	100		104.9	99.2	--	91.9[a]

a. At the time these experiments were performed, it was not known that incubation of vermiculite could cause a decrease in CO concentration with subsequent exposure of the vermiculite. Thus, vermiculite controls were not moistened and kept in the greenhouse for duration of plant growth.

been due to normal soil uptake variation. With ambient carbon monoxide concentration, however, slight increases in carbon monoxide occurred in the presence of peppers and geraniums in support media.

Marine Specimens

Changes in carbon monoxide levels in the presence of marine algae at 10°C (2 experiments, each species) are presented in Table 3.

Table 3

EFFECT OF SALTWATER ALGAE ON THE
CONCENTRATION OF CARBON MONOXIDE AT 10°C

Specimen	Wet Weight (grams)	Carbon Monoxide Concentration (ppm) 0 Hr	24 Hr	48 Hr
Cystoseira	85	111.0	108.1	102.6
	780	113.9	108.3	104.3
Egregia	364	110.6	110.6	108.7
	1668	113.9	119.2	130.3[a]
Macrocystis	285	111.3	106.1	99.4
	1059	111.3	108.3	102.9
Control (seawater)	11.5[b]	110.6	105.6	99.4
		112.6	108.3	107.4

a. This sample of Egregia was stored in a refrigerator overnight, rather than in filtered seawater.
b. Liters.

Although a slight decrease in carbon monoxide concentration occurred in the presence of algae in seawater, similar decreases occurred in the seawater controls. At an initial concentration of 110 ppm carbon monoxide, there was

neither a marked reduction in, nor evolution of, carbon monoxide by these species at 10°C, suggesting that algae are neither a source nor a sink for carbon monoxide at this temperature. (The one exception is the sample of Egregia that was refrigerated out of seawater by the suppliers overnight.)

These results do not support those obtained by Loewus and Delwiche,[6] who found that fresh fronds, stipes, and macerated tissues of Egregia menzies in phosphate buffer evolved carbon monoxide in easily detected concentrations. The fresh tissue used by these workers, however, was stored at -10°C prior to use, which may have altered normal metabolic processes for carbon monoxide. Evidence that storage conditions may influence the character of carbon monoxide metabolism in Egregia is apparent in the results presented in Table 3. The sample of Egregia that had been mistakenly refrigerated prior to delivery demonstrated carbon monoxide evolution, while nonrefrigerated tissue of the same age and harvest did not demonstrate a net evolution of carbon monoxide.

At 19.5°C, a slight trend toward carbon monoxide removal by Macrocystis and Egregia was apparent. See Table 4. This removal amounted to about 7 ppm for a 48-hour period for Macrocystis (5.13×10^{-8} g/g tissue/hr) and 15 ppm for Egregia (11×10^{-8} g/g tissue/hr) for a similar period.

Table 4

EFFECT OF SALTWATER ALGAE ON CONCENTRATION OF CARBON MONOXIDE AT 19.5°C

Specimen	Wet Weight (grams)	Carbon Monoxide Concentration (ppm) 0 Hr	24 Hr	48 Hr
Macrocystis	431	96.5	92.0	88.0
Egregia	578	97.4	93.9	81.3
Control (seawater)	11.5[a]	100.1	95.7	98.4
		98.2	98.9	97.0

a. Liters.

V DISCUSSION

Relatively high levels of carbon monoxide in balance air rapidly disappeared when continually recirculated over nonsterile soil. Moistened vermiculite that had been exposed to ambient air conditions for several weeks also caused comparable depletion of carbon monoxide. Depletion rates increased markedly with increasing temperatures. Nonsterile soil and vermiculite containing higher plants demonstrated the carbon monoxide depletion, and a trend toward slight decreases in carbon monoxide was observed above marine algae in seawater.

On the other hand, carbon monoxide did not decrease in the presence of steam-sterilized soil or vermiculite. In fact, at ambient carbon monoxide concentrations, exposure of sterilized soil resulted in slight increases in carbon monoxide concentrations.

Apparent uptake of carbon monoxide by soil may be explained hypothetically by either of two mechanisms: (1) adsorption or binding by nonliving soil particles and plant debris or (2) active utilization or binding by the soil microflora. The evidence obtained during this research strongly suggests that assimilation by soil microorganisms is the most likely mechanism. The major evidence supporting this conclusion is the prevention of carbon monoxide depletion by steam sterilization in two structurally unrelated media and the increase in disappearance rates with increasing temperatures. Increasing temperatures within certain physiological limits not only would be expected to accelerate the rate of biological carbon monoxide reactions but also to increase the rate of reproduction of such organisms, and thereby increase proportionately the volume of cells and number of sites for carbon monoxide reactions. Moreover, any active uptake, carbon monoxide reaction or binding mechanisms employed by soil microorganisms would be heat labile, and consequently would be destroyed by temperatures prevailing

during steam sterilization (121°C). On the other hand, if reduction in carbon monoxide concentrations was due to adsorption on the surface of soil components, disappearance rates would decrease rather than increase with increasing temperatures within the range studied. The release of bound carbon monoxide by the thermal degradation of organic soil components is postulated as an explanation for the slight net increase of carbon monoxide over sterilized soil.

The natural soil complement of the mixture used in the described tests originated within the Los Angeles area, and the mixture itself was continuously exposed to ambient atmospheric conditions characteristic of that area prior to use. In view of the relatively high ambient concentrations of carbon monoxide in the air in the Los Angeles basin, it is possible that induction or increase of carbon monoxide reactive mechanisms or population selection pressure was exerted on soil microflora present both during and prior to the storage period. The soil aliquots tested, therefore, may be relatively effective specimens with regard to capability to remove carbon monoxide from test atmospheres. Conversely, since soil samples removed from areas not characterized by high atmospheric levels of carbon monoxide might be expected to be less effective in assimilating atmospheric carbon monoxide, it is difficult to project the data obtained to a quantification of the role of soil microflora in carbon monoxide uptake on a worldwide basis. Any estimate so obtained would have to be considered as being liberal, and in all probability would exceed that actually occurring on a worldwide basis. Using the minimal disappearance rate of carbon monoxide obtained in our experiments (1.7 ppm per hr) an estimate of 2.06×10^{15} grams per year can be made. See Appendix A.

The mechanism(s) of land plant removal of carbon monoxide probably differs from that of soil microorganisms. In the present series of experiments, a rapid decrease in carbon monoxide concentration in the presence of plants in support medium was observed, but a similar decrease in the presence of support medium

alone was observed. Thus, all these depletions could easily be accounted for by the support medium alone. Capacity of land plants as a carbon monoxide sink may equal that of soil microorganisms on a worldwide basis; however, the effects of the two are not easily separated. Because of the large volume of plant material throughout the world, only a small carbon monoxide decrease per gram of tissue would account for annual carbon monoxide disappearance (see Appendix A). This could be easily masked by a combination of factors such as rapid carbon monoxide removal by support medium alone or alteration of the soil microbial population by the presence of plants.

Marine algae appear to have a mechanism for carbon monoxide removal that is easily disrupted by environmental stress. Although rates of disappearance of carbon monoxide were nominal in specimens stored in near-normal environmental conditions, abnormal storage conditions resulted in an increased level of carbon monoxide. Further experiments should therefore be conducted at conditions as close to physiological as possible, since overnight storage under refrigeration induced an increase in carbon monoxide concentration, which was contrary to findings with specimens not so treated. Loewus and Delwiche, who stored specimens under abnormal conditions ($-10^{o}C$) and tested their specimens in phosphate buffer, also found evolution of carbon monoxide. It may be that the carbon monoxide removal mechanism(s) is intimately associated with tissues having a continuous history of normal metabolic activity and is easily damaged or destroyed by sudden or extreme changes in environmental conditions, whereas the carbon monoxide evolution mechanism(s) is more resistent to environmental stress. The use of experimental, altered media should be avoided if the normal metabolic character of the specimen is to be determined. As a preliminary estimate of carbon monoxide removal under normal conditions, marine algae could account for an annual disappearance of 4.08×10^{11} grams of carbon monoxide per year (see Appendix A).

If the roles of these elements of the biosphere are to be better defined as sinks for carbon monoxide, several definitive types of work are needed. Because of the multiplicity of types of microorganisms in the soil, specific microorganisms responsible for carbon monoxide disappearance must be identified for capacity to remove carbon monoxide. A corollary series of experiments investigating soil types and their respective microfloral balances for their efficiency in removing carbon monoxide would enable a more meaningful worldwide estimate of soil carbon monoxide capacity.

The evaluation of aseptically grown and tested plants would enable the separation of plant effects from those of microflora in the support medium. Evaluation of marine algae under normal physiological conditions not only would provide a better estimate of the marine plant role in carbon monoxide removal but, perhaps, also aid in the elucidation of other carbon monoxide metabolic balances.

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Appendix A

CARBON MONOXIDE UTILIZATION PROJECTIONS

SOIL UTILIZATION PROJECTION

Based on our lowest figure of experimental disappearance of carbon monoxide in the presence of unsterilized soil, it can be calculated that soil can more than account for the annual carbon monoxide disappearance on a worldwide basis at 2.06×10^{15} grams/yr.

5 cu ft = 8640 cu in. = capacity of environator

512 cu in. occupied by soil (4 Pyrex containers per environator)

1.7 ppm/hr = experimentally determined disappearance rate of carbon monoxide

57.506×10^{6} sq mi = earth land surface

R = 82.057

p = 1 atm

v = 5.434 cc

T = $295.5^{\circ}K$

8640 cu in. (capacity of environator) - 512 cu in. (area occupied by soil) = 8128 cu in. (actual gas space)

1.7 ppm/hr carbon monoxide disappearance = 40.8 ppm/24 hr

8128 cu in. (actual gas space) x 16.39 cc/cu in. = 133,218 cc/chamber

$$\frac{40.8 \text{ ppm}}{1 \times 10^{6}} = \frac{X}{1.332 \times 10^{5}}$$

X = 5.434 cc, actual CO in chamber

For changing cubic centimeters of gas to grams of gas:

$pv = nRT$

$$\frac{1 \times 5.434 \times 28}{82.057 \times 295.5^{\circ}} = 0.006277 \text{ grams carbon monoxide per 256 sq in./24 hr}$$

1 sq mi = 4.0145×10^9 sq in. ÷ 256 sq in./environator = 1.568×10^7

$1.568 \times 10^7 \times 6.277$ mg/chamber = 9.84×10^7 mg/sq mi

Since 57.506×10^6 sq mi = earth land surface

9.84×10^7 mg/sq mi x $57.506 \times 10^6 = 566 \times 10^{13}$

or

5.7×10^{15} mg/day x 365 = 2060×10^{15} mg

or

2.06×10^{15} grams/yr

MARINE PLANT USAGE PROJECTION

Based on screening experiments of marine algae, the possible disappearance of carbon monoxide due to marine plant life might be projected on an annual basis as 4.08×10^{11} grams/yr.

Mol wt of carbon monoxide = 28

Macrocystis weight = 431 grams

Experimental disappearance rate = 7 ppm CO/48 hr

5 cu ft = size of environator

T = 292.5°K

R = 82.057

Seawater vol = 11.5 liters

1×10^9 tons algae produced per year

5 cu ft x 28.32 ℓ/cu ft = 141.6 liters (capacity of small environators)

141.6 liters - 11.5 liters = 130.1 liters (actual gas capacity of environators)

A 7 ppm change = 0.9107 cc in 130,100 cc capacity

$$pv = nRT$$

$$n = \frac{(28)(1)(0.9107)}{(82.057)(292.5)} = 0.001062 \text{ grams/48 hr/431 grams}$$

$$\frac{2000 \text{ lb} \times 454 \text{ grams} \times 1 \times 10^9 \text{ tons} \times 1.062 \times 10^{-3}}{431 \text{ grams} \times 2 \text{ days}} = 1118.7 \times 10^6 \text{ grams/day}$$

1118.7×10^6 grams/day x 365 days = 4.08×10^{11} grams CO/yr that could be used by algae at this rate

LAND PLANT USAGE PROJECTION

Assuming 0.5 lb of plant material per experimental unit, and 1.267×10^{16} grams of total land plant material, the rate of disappearance of carbon monoxide per environator per hour that would be necessary for plants to act as a carbon monoxide sink can be calculated. This equals 2.76 ppm/hr.

2.1×10^{14} grams CO produced by urban activities per year

1.267×10^{16} grams of land plant material

225 grams of plant material per environator

22.5°C temperature

133,218 cc actual gas space per environator

p = 1 atm

R = 82.057

T = 295.5°K

$$\frac{2.1 \times 10^{14} \text{ grams}}{1.267 \times 10^{16} \text{ grams} \times 365 \text{ days} \times 24 \text{ hr}} = 1.89 \times 10^{-6} \text{ grams CO/hr/grams of plant material}$$

$1.89 \times 10^{-6} \times 225$ grams of plant material $= 4.25 \times 10^{-4}$ grams CO/hr per environator

$pv = nRT$

$$(1 \text{ atm})(v) = \frac{4.25 \times 10^{-4}}{28} (82.057)(295.5^{\circ}\text{K})$$

v = 0.3677 cc/hr per environator

or

$$\frac{0.3677 \text{ cc}}{133{,}218 \text{ cc of space}} = 2.76 \text{ ppm carbon monoxide should disappear from each box per hour}$$

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